IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(Case No. 05-1083)

In the Application of:	)	
	)	
Vincent Cool, et al.	)	
	)	Examiner: Ronald T. Niebauer
Serial No.: 10/562,998	)	
	)	Group Art Unit: 1654
Filing Date: May 2, 2006	)	
	)	Confirmation No. 3592
For: Process for Supported Phase Synthesis	)	

DECLARATION OF LUCIANO FORNI UNDER 37 CFR 1.132

Commissioner for Patents
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Alexandria, VA 22313-1450

I, Luciano Forni, do hereby declare and say as follows:

1. I am an inventor of the claimed subject matter of the above-identified application.

2. I studied Organic Chemistry at the Université Libre de Bruxelles for 4 years, finishing my studies with a Master of Science degree. Upon graduation, I worked for seven years at Christiaens S.A. in research and development. I then worked for 26 years at UCB-Bioproducts, a subsidiary of UCB, S.A., where I specialized in peptide synthesis. As part of this work, I supervised an R&D peptide laboratory composed of 4 PhDs, 12 technicians and 2 workers. I also supervised the first mid-scale commercial production of peptides. Since 2006, I have worked at Lonza - Braine S.A. (formerly UCB-Bioproducts) in the department of Process Optimization and Technology where I currently supervise 3 PhDs and 10 technicians in work relating to peptide synthesis.

During the course of my career as a peptide chemist, I gained intimate knowledge of the methodology of both Homogeneous Phase Peptide Synthesis (HPPS) and Solid Phase Peptide Synthesis (SPPS). I worked with each process on the laboratory scale, pilot plant

scale, and full production scale. I first started working with SPPS in the 1980's using Boc/Bzl. When Fmoc protected amino acids became commercially cost-effective, I participated in the development of Fmoc SPPS technology for the production of long and short peptides. In particular, I was the Head of the Technology Development Department during the development of Fmoc/tBu SPPS technology on a production scale. In one aspect of SPPS technology, we pioneered the practice of in-line monitoring of the Fmoc deprotection step using proprietary technology. I also supervised various improvements of SPPS chemical processes resulting in more efficient synthesis with shorter reaction times and fewer steps, while avoiding unwanted side reactions. I have supervised the investigations of resins that previously had not been used in SPPS, and optimized the selection of solvents. In my role as Head of Process Development and Technology, I have been called upon to define appropriate synthetic routes for various peptide products, requiring an in-depth knowledge of HPPS, SPPS, side chain protecting groups, resins, and convergent and non-convergent synthetic strategies.

3. As a result of my education and my many years of professional experience as a peptide chemist and supervisor of several peptide research and development laboratories, I consider myself to be skilled in the art of peptide chemistry and peptide synthetic techniques, including solid phase peptide synthesis ("SPPS"), and qualified to make the statements herein.

4. As one skilled in the art of SPPS, I am aware that the process generally comprises the steps of cleaving a protecting group such as Fmoc from a peptide which is bound to a solid phase to de-protect the peptide, coupling a protected amino acid to the de-protected peptide, then cleaving the protecting group from the amino acid that was just coupled to the peptide, such that another group can be coupled. In this manner, peptide chains of desired amino acids in a desired sequence can be synthesized. Cleavage of the protecting groups typically occurs under basic conditions.

5. It is known that after the step of cleaving the protecting group from the bound peptide, the reaction medium will contain unprotected amino acids on the ends of peptides bound to a solid phase, as well as unbound protecting groups and possibly other reaction by-products. It is known that it is desirable to thoroughly wash the peptides bound to the solid-state support, to remove the unbound protecting group and by-products before the next coupling step. This washing step corresponds to step (b) of present claims 3 and 8.

6. It is further known that after the step of coupling a desired protected amino acid to the deprotected amino acid, the reaction medium may contain excess protected amino acid and other reaction by-products. It is known that it is desirable to thoroughly wash the peptides bound to the solid-state support, to remove all the unbound protecting group and by-products before the next cleaving step. This washing step corresponds to step (d) of the present claim 8.

7. The invention of the present patent application relates to improvements in methods of solid state syntheses of peptides, and in particular to our discovery that the use of certain phosphonium, sulfonium, and quaternary ammonium salts during synthesis can significantly improve the efficiency of the wash steps described above.

8. In one aspect of the present invention, this is achieved by the use of the claimed salts, inter alia, the benzyltrimethylammonium hydroxide, in one of these two washing steps.

9. We have found that, surprisingly, the use of these claimed salts in one of these two washing steps reduces the number of necessary washing cycles in these two washing steps. This reduction represents a considerable advantage during large scale production of peptides with SPPS by reducing the cycle times and the amounts of solvent, resulting in both a faster process and a lower cost.

10. The disclosure of the present application in examples 3 and 4 demonstrates that the number of washing cycles after the coupling step c) have been greatly reduced by using benzyltrimethylammonium hydroxide in the washing steps d).

11. When the benzyltrimethylammonium hydroxide is used in the washing step b), which is performed after the cleaving step a), the Fmoc groups have already been cleaved in step a) and are therefore no longer attached, and consequently, the benzyltrimethylammonium hydroxide in the wash medium cannot induce any more cleaving.

12 When the benzyltrimethylammonium hydroxide is used in the washing step d), which is performed after the coupling step c), the Fmoc groups are all present. But any cleaving, which is induced be the basic benzyl trimethyl ammonium hydroxide during the washing step

d), does not interfere with any subsequent reactions, since in the subsequent cleaving step a) in the next SPPS cycle, the Fmoc groups are cleaved anyway.

13. I have reviewed each of the Rink (U.S. 5,004,781), Mihala, and Merrifield references cited against the present application. Each one of these references teaches the use of the benzyltrimethylammonium hydroxide or of an ammonium salt in a certain reaction step, this reaction step occurring either during SPPS or even before SPPS:

a) Mihala teaches the use of an ammonium salt as a coupling additive together with a coupling agent in the coupling step of SPPS. He uses the combination to improve this reaction.

b) Merrifield in combination with Finger also teaches the use of benzyltrimethylammonium hydroxide in the coupling step, which is called "acylation" by Merrifield. Again, he uses the ammonium salt to improve this reaction.

c) Rink teaches the use of benzyltrimethylammonium hydroxide in the reaction step, which is the cleaving of the protecting group from the handle after attaching the handle to the resin. This is a reaction step occurring in the preparation of a resin, which thereafter can be used in SPPS, i.e. it is a reaction step even before the SPPS.

14. In particular with respect to the Rink reference I note the following:

a. At column 3, line 34 – column 5, line 25, Rink discloses a process for making a resin which can be used as a support in peptide synthesis. The resin can carry a group -NH-W in which NH is an amino group and W is an amino-protecting group. The group W must be cleaved to deprotect the amino group so that the amino group on the resin can serve as a site for peptide synthesis. Rink states that this deprotecting step can be accomplished with "a solution of a tertiary or, preferably, secondary, open-chained or cyclic amine…" (col. 5, lines 9-10). Rink states that it is also possible to use benzyltrimethylammonium hydroxide to deprotect the amino group on the resin (column 5, lines 19-25).

b. At column 5, line 26 – column 9, line 23, Rink discloses a process of using the resin to synthesize peptides. At col. 8, lines 43-46, the reference states that N-terminal protecting groups can be removed with inorganic or organic bases, especially with tertiary or secondary amines. In this discussion the reference does not mention the use of quaternary amines.

c. I have reviewed the examples at column 12, line 41 – column 19, line 63. None of these examples recites the use of a salt in any wash step.

From the foregoing it is my understanding that Rink teaches the use of benzyltrimethylammonium hydroxide ONLY during the amino group deprotection steps of a handle group coupled to a resin. This disclosure does not mention the use of ammonium salts during resin washings after the coupling and deprotection steps.

16. As one skilled in the art of SPPS methods, I learn from these three references, that in certain reaction steps of SPPS or before SPPS, either benzyl trimethyl ammonium hydroxide or a certain ammonium salt was used in order to enhance this specific reaction.

17. The combined teaching of the three references is the use of benzyl trimethyl ammonium hydroxide (only two references) or of an ammonium salt (all three reference) in one of three specific reaction steps, these three reaction steps occurring during SPPS (two of the references) or before SPPS (one reference).

18. As one skilled in the art of SPPS methods, these three references, either alone or in combination, do not teach or suggest the use of benzyltrimethylammonium hydroxide or any other salt in one of the two washing steps b) or d).

19. As one skilled in the art of SPPS methods, these three references, either alone or in combination, do not teach or suggest that the use of benzyltrimethylammonium hydroxide or of any other salt in one of the two washing steps b) or d) will reduce the number of necessary wash cycles within each wash step.

I hereby state that I have been warned that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. 1001), and that such willful false statements may jeopardize the validity of the application or document or any registration resulting therefrom, and I declare that all statements made of my own knowledge are true; and all statements made on information and belief are believed to be true.

Luciano Forni

Date: November 24, 2010